Petroleum HPV

**201-15019**

December 15, 2003

The Honorable Michael O. Leavitt, Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116



Attention: Chemical Right-to-Know
HPV CONSORTIUM
Lubricating Grease Thickeners Test Plan and Robust Summary

Dear Administrator Leavitt:

The American Petroleum Institute, on behalf of the Petroleum HPV Testing Group, is pleased to submit the Lubricating Grease Thickeners Test Plan and Robust Summary. Our consortium has chosen not to use the HPV Tracker system for submission of our test plans due to the complexity of petroleum substances categories and the associated test plans. We are therefore submitting this test plan, as well as the robust summary, directly to EPA to make available for public comment.

Electronic copies of the test plan (in .pdf format) and robust summary (in .pdf format) are accompanying this letter via email to the EPA HPV electronic submission addresses and to the individuals listed below, including Mr. Charles Auer. Within the next two weeks, an electronic version of the robust summary as an IUCLID export file will also be sent to the EPA HPV email address.

Please feel free to contact me (202-682-8344; twerdokl@api.org) or Tom Gray (202-682-8480; grayt@api.org) with any comments or questions you may have regarding this submission.

Sincerely,

Lorraine Twerdok, Ph.D., DABT
Administrator, Petroleum HPV Testing Program

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**201-15019A**

# HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM

## TEST PLAN

**Fatty Acids, Lithium & Calcium Salts used as Grease Thickeners**



**Submitted to the US EPA**

**by**

**The Petroleum HPV Testing Group**

**www.petroleumhpv.org**

**Consortium Registration**

**December 31, 2003**

# TEST PLAN

# GREASE THICKENERS CATEGORY

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## PLAIN LANGUAGE SUMMARY

The lithium and calcium salts of fatty acids in this category are used by the lubricants industry to thicken greases. For this use they are not synthesized as the "pure" compounds but are made only in the presence of oil. One or more fatty acids is dissolved in mineral oil and then a caustic such as calcium hydroxide or lithium hydroxide is added. The caustic and fatty acid molecules react to form the insoluble metal salt of the fatty acid. The resulting compounds gel the mineral oil into a functional grease. Greases typically contain from approximately 1- 14 % thickener by mass.

The calcium and lithium salts of fatty acids used as grease thickeners in this category are considered very low in toxicity based on extensive use in industry without reports of significant adverse effects for many decades. The fatty acids from which the salts are made are either edible or similar in structure to edible fatty acids. The salts formed in the presence of mineral or synthetic oils are not readily bioavailable due to size and limited solubility in the grease matrix.

Results from testing lithium fatty acid salts, fatty acid salts compositionally similar to salts in this category (eg. magnesium stearate) and greases containing thickeners from this category, demonstrate that these materials are not acutely toxic by the oral or dermal route, are not irritating to the eyes or skin and do not induce skin sensitization. Repeat dose studies in rats by the oral route (Mg stearate – 3 months in diet; castor oil – 13 wks in diet; lithium complex grease –90 days by oral gavage), or with dermal treatment (lithium complex grease – 28 or 90 days) did not show any significant adverse effects. Treatment with a lithium grease dermally for 2 years did not cause skin cancer in C3H mice. Mutations were not induced in bacterial assays by fatty acids used to make salts in this category. Soluble lithium salts were not mutagenic *in vitro*, and slight chromosomal effects occurred only from a very high dose of lithium citrate administered intraperitoneally. Considering, along with these data, the low solubility of salts of fatty acids in the grease thickeners category, the compounds are not unlikely to be mutagenic. No developmental or reproductive toxicity assays are available for lithium salts of fatty acids in this category. Magnesium stearate (structurally similar to calcium stearate) did not induce developmental effects in orally treated pregnant rabbits. Two calcium salts in this category are not considered candidates for SIDS testing under the HPV Challenge Program.

Substances in the grease thickeners category can be considered environmentally innocuous due to their origin from alkali metals and edible fats and oils (or similar fatty acids) and their low water solubility. The hydrocarbon components of the greases have little or no tendency to

partition into air, are not susceptible to hydrolysis or direct photolysis under environmental conditions, and will partition primarily to soil and sediment. Fatty acids are known to be used by microorganisms as a source of energy, and the calcium and lithium salts of fatty acids are biodegradable. Technical discussions and computer modeling will be used to address physicochemical and environmental fate endpoints.

A technical discussion will be prepared for the robust summary that describes the potential for substances in this category to affect aquatic organisms. Due to the process of creating the fatty acid salts “in situ” within the oil matrix, exposure to aquatic organisms by these substances is unlikely. Therefore, aquatic toxicity testing of these products would not further the understanding of their aquatic hazard, and therefore such testing is not warranted. Aquatic toxicity data on the dissociation products of these grease thickeners, i.e., lithium, calcium, and the associated fatty acids, will be included in the technical discussion along with relevant physicochemical information affecting their distribution in aquatic environments.

The test plan proposes the performance of a dermal reproductive/ developmental toxicity screening test (OECD 421) in rats using a greases thickened with approximately 12% lithium 12-hydroxystearate and no additives present. Results of this study combined with currently available data on calcium and lithium salts and compositionally similar salts of fatty acids, as well as test results from greases thickened with these salts are adequate to complete the hazard profile for materials in this category.

## Description of the Grease Thickeners Category

Fatty acids, calcium & lithium salts are used by the lubricant industry to thicken greases. Most lubricants are mixtures but greases are one of the few types of lubricants that involve an actual chemical reaction during their manufacture. Although a few greases are thickened with viscous petroleum products similar to asphalt, most are made by the formation of a "soap" within a mineral oil matrix. One or more fatty acids is dissolved in mineral oil and then a caustic such as calcium hydroxide or lithium hydroxide is added. The caustic and fatty acid molecules react to form the insoluble metal salt of the fatty acid. The resulting compounds gel the mineral oil into a functional grease. Greases containing these compounds typically contain from 1-14% thickener on a mass basis.

If fatty acids or triglycerides are reacted with caustic outside of a mineral oil matrix, the resulting compounds are called soaps, hence the use of this term relative to grease thickeners of this type. Water or methanol is usually formed during the reaction depending on whether the fatty acid or its methyl ester, respectively, was used as a starting reactant. Additional performance additives such as extreme pressure agents and antioxidants may be added to a grease before or after thickening. Some greases are thickened with the lithium salt of two different fatty acids and these may be called "lithium complex" greases. Greases thickened with aluminum, calcium or lithium soaps have been widely and safely used for several decades.

The fatty acids used to make greases are derived from edible animal fats or vegetable oils. The fatty acids used as starting materials in this category are mostly monocarboxylic acids and include stearic acid ($C_{18}$), 12-hydroxystearic acid, docosanoic acid ($C_{22}$), hydrogenated castor oil (comprised of ricinoleic and similar acids, $C_{18}$), and methyl esters of oxidized hydrocarbon waxes ($C_{\geq 18}$). One lithium salt of a dicarboxylic acid (azelaic, $C_9$) is included in the category as it is commonly used in lithium complex greases. Azelaic acid (nonanedicarboxylic acid) is manufactured from ricinoleic acid (castor oil).

The metal salts in this HPV category are lithium or calcium. There are 11 CAS numbers in the HPV Grease Thickeners category. Some of the compounds were included in the original list sponsored by the API (1999) based on the EPA 1990 IUR, and other non-HPV materials have been added due to their structural similarity. The 11 category members are listed in Table 1.

**Table 1: HPV Grease Thickeners Category**

| CAS # | Name | Synonym |
|---|---|---|
| 3159-62-4 | Octadecanoic acid, 12-hydroxy-, calcium salt (2:1) | Calcium 12-hydroxystearate |
| 38900-29-7 | Nonanedioic acid, dilithium salt | Dilithium azelate |
| 4485-12-5 | Octadecanoic acid, lithium salt | Lithium stearate |
| 4499-91-6 | Docosanoic acid, lithium salt | Lithium docosanoate |
| 53422-16-5 | Octadecanoic acid, 12-hydroxy-, methyl ester, lithium salt | Lithium 12-hydroxystearate (same as 7620-77-1) |
| 64754-95-6 | Castor oil, hydrogenated, lithium salt | |
| 68783-36-8 | Fatty acids, $C_{16-22}$, lithium salts | |
| 7620-77-1 | Octadecanoic acid, 12-hydroxy-, monolithium salt | Lithium 12-hydroxystearate (same as 53422-16-5) |
| 1592-23-0 | Stearic acid, calcium salt | Calcium stearate |
| 64755-01-7 | Fatty acids, tallow, calcium salts | |
| 68603-11-2 | Hydrocarbon waxes, petroleum, oxidized, Me esters, calcium salts | |

Two compounds in the category have different CAS numbers and slightly different names but are chemically the same. Lithium 12-hydroxystearate is formed from both the methyl ester of 12-hydroxy octadecanoic acid and from the acid itself. If the methyl ester is used, then methyl alcohol is formed as a byproduct instead of water.

Two other compounds in this category [Stearic acid, calcium salt (CAS 1592-23-0) and Fatty acids, tallow, calcium salts (CAS 64755-01-7)] are considered adequately characterized relative to the Screening Information Data Set. These two compounds are designated “1” and the EPA has concluded that the “Chemical is not considered a candidate for testing under the HPV Challenge Program, based on preliminary EPA review indicating that testing using the SIDS base set would not further our understanding of this chemical’s properties” (EPA HPV Challenge Program Chemical List).

Typical Properties

Table 2 summarizes the typical physical and chemical properties that characterize the calcium and lithium salts in this category and some closely-related compounds.

**Table 2: Physicochemical properties of fatty acid salts used as grease thickeners and related compounds**

| Compound | Carbon number | Molecular wt. | Melting point (°C) | Water solubility |
|---|---|---|---|---|
| **Category members:** | | | | |
| Calcium 12-hydroxystearate | 18 | 640 | | |
| Calcium stearate | 18 | 608 | 179 | 0.004g/100cc @15$^0$C |
| Fatty acids, tallow, calcium salts | 14-18 | > 490 | | |
| Hydrocarbon waxes, petroleum, oxidized, Me esters, calcium salts | > 18 | > 600 | | |
| Lithium stearate | 18 | 291 | 220-221 (CIR) | "insoluble" (CIR) |
| Lithium 12-hydroxystearate | 18 | 307 | | |
| Castor oil, hydrogenated, lithium salt | > 16 | > 260 | | |
| Fatty acids, $C_{16-22}$, lithium salts | 16-22 | ~263-347 | | |
| Lithium docosanoate | 22 | 347 | | |
| Dilithium azelate | 9 | 202 | | |
| **Related compounds:** | | | | |
| Sodium stearate | 18 | 308 | | "slowly soluble" |
| Magnesium stearate | 18 | 591 | 88 ?; 132 | 0.003g/100cc @15$^0$C |
| Zinc stearate | 18 | 632 | 130 | "insoluble" |
| Sodium oleate | 18 | 305 | 232-235 | 10g/100cc@ 12$^0$C |
| Potassium oleate | 18 | 322 | 235-240 | 25g/100cc cold water |
| Sodium palmitate | 16 | 278 | 270 | "insoluble" |

## CATEGORY JUSTIFICATION AND TEST MATERIAL DESCRIPTION

All of the compounds in this category are the lithium or calcium salt of similar fatty acids. All of the fatty acids are similar in size (14 carbons long or longer) with the exception of nonanedioic acid (azelaic), which contains 9 carbons. Stearic acid is found in food, and similar to castor oil, is used in cosmetics and pharmaceuticals. These and the other fatty acids are similar in chemical characteristics. Several of these fatty acids are HPV chemicals sponsored by other chemical manufacturers. All of these salts of fatty acids synthesized "in situ" in a mineral

oil matrix and their biological activity is low because of high molecular weight and insolubility. Therefore testing from one compound can be extrapolated to likely effects of other category members.

The material selected for this test plan is a lithium salt thickened grease synthesized from USP white mineral oil and approximately 12% lithium 12-hydroxystearate. Since for lubricant purposes, fatty acid salts are only formulated in the presence of petroleum oil, this lithium grease thickener will be tested in a petroleum oil matrix.

## EVALUATION OF EXISTING HEALTH EFFECTS DATA AND PROPOSED TESTING

### Introduction

The calcium and lithium salts of fatty acids used as grease thickeners in this category are considered very low in toxicity based on their extensive use without reports of significant toxicity for many decades. A very large population of workers has had frequent dermal exposure while using greases for the lubrication of bearings and other moving parts in virtually every segment of transportation and industry.

The compounds in this category are made from fatty acids that are either edible or similar in structure to edible fatty acids. These fatty acids in their free state are readily absorbed from the gastrointestinal tract and readily metabolized. The calcium and lithium salts that are formed in the presence of mineral or synthetic oils, however, are not readily bioavailable and their function is to maintain the oils in a gel-like state in contact with the surfaces being lubricated. High resistance to water wash-out is a desirable trait of most greases.

Calcium is common in all living systems and is essential to life. Lithium ion is used pharmacologically to treat bipolar disorder and is toxic at higher plasma concentrations. Lithium has significant bioavailability only when administered as a partially soluble salt such as lithium carbonate. Both the calcium and lithium salts of the fatty acids in this category, especially when present in an oil matrix, have extremely low bioavailability.

Toxicological data on several stearic acid compounds including the aluminum, calcium, and lithium salts, were reviewed by the Cosmetic Ingredient Review (CIR) Panel (1982).

Toxicological studies of calcium and lithium salts of fatty acids in their pure form and studies with greases thickened with these salts are both considered relevant to an assessment of their hazards.

**Study Review and Evaluation**

Results of studies on materials in this category and materials similar to those in this category are summarized in this section. Detailed study information is available in the Robust Summaries organized in the IUCLID data set format employed by the European Union (Appendix 1). The currently available data submitted to the HPV program and any additional testing will be developed with the goal of facilitating international harmonization of hazard and risk characterization worldwide.

**Acute Toxicity**

Calcium salts of fatty acids:

There are no acute toxicity data for the calcium salts *per se*. However, data may be extrapolated from available information on magnesium salts such as Mg stearate (oral LD50 > 10 g/kg; CIR, 1982). Ca stearate is cleared as a direct food additive and is considered GRAS (Generally Recognized as Safe).

Mg stearate was not irritating when tested on the skin or in the eyes of rabbits (CIR, 1982).

Lithium salts of fatty acids:

The oral LD50 of Li stearate in the rat is > 5 g/kg (CIR, 1982). A lithium complex grease containing 13.1% lithium 12-hydroxystearate and 2.6% dilithium azelate had an oral LD50 in the rat > 5 g/kg (Pharmakon, 1994a). This same grease had a dermal LD50 in the rabbit > 3 g/kg (Pharmakon, 1994b) and did not induce acute skin or eye irritation potential (Pharmakon, 1994 c,d).

Another lithium complex grease containing 8.8% lithium hydroxystearate and 1.8% dilithium azelate was tested for skin sensitization potential using a Buehler assay. This grease, which also contained several performance additives, was negative for sensitization (Pharmakon, 1997).

**Summary:** The data available demonstrate that the substances in this group are not acutely toxic by either the oral or dermal routes. They are non-irritating to the skin and eye and are not sensitizing to the skin. Both calcium and lithium stearates have been safely used in cosmetics and lithium stearate has been used in baby powders to aid in water repellency and oil absorbency (CIR, 1982). **There are sufficient data to characterize this group of substances for acute toxicity. No additional testing is proposed.**

**Repeated Dose Toxicity**

Calcium salts of fatty acids:

No 28-day or longer studies are available on the calcium compounds in this category. However, data may be extrapolated from a study with magnesium stearate. Rats were fed Mg stearate up to 20% in the diet for 3 months (Sondergaard et al., 1980). There were no significant histopathologic changes. The NOEL was 5% (~ 2500 mg/kg/day).

Lithium salts of fatty acids:

A lithium complex grease containing 8.8 % lithium 12-hydroxystearate and 1.8% dilithium azelate was tested in a 90-day oral study (Huntingdon, 1977) and in 28-day and 90-day dermal studies (Huntingdon, 1997a,b) in the rat. This grease also contained performance additives at a total concentration of about 7 percent. There were no significant adverse effects after 90 days of oral dosing with the grease at 1000 mg/kg/day or after 2100 mg/kg/day dermally. Therefore, the NOEL for lithium 12-hydroxystearate was 88 mg/kg/day orally and 185 mg/kg/day dermally. The NOEL for dilithium azelate was 18 mg/kg/day orally and 38 mg/kg/day dermallly.

Another study that is relevant to both the calcium and lithium salts in this category is a 13-week dietary study in rats and mice with castor oil conducted by the National Toxicology Program (1992). The predominant fatty acid in castor oil is ricinoleic acid (12-hydroxy-*cis*-9-octadecenoic acid) while 12-hydroxystearic acid is 12-hydroxyoctadecanoic acid. Diets containing up to 10% castor oil had no adverse effects.

Grease containing 7.5 percent lithium 12-hydroxystearate was tested in a chronic skin-painting study with 50 male and 50 female C3H/HeJ mice (Barkley and Stemmer, 1984). This grease also contained performance additives at a total concentration of about 12 percent. The total tumor incidence was 3/100. Since a tumor incidence of at least 4 percent is considered positive, the results of this assay were negative for carcinogenicity.

**Summary:** Repeated dose studies including a chronic skin-painting study have been conducted with greases containing lithium 12-hydroxystearate and/or dilithium azelate. Studies have been conducted with magnesium stearate, which is closely related to calcium stearate. A study has been conducted with castor oil (mostly ricinoleic acid) which is closely related to the larger fatty acids used to make the salts in this category. Results of reported studies on similar compounds are used for read-across to the grease thickeners in this category. **No additional testing is proposed.**

**Genotoxicity (*in vitro* and *in vivo*)**

No studies have been published/reported on the genotoxicity of either calcium or lithium salts in this category. However, castor oil and magnesium stearate have been tested in the Ames test and were negative for mutagenicity (NTP, 1992; Litton Bionetics, 1976, respectively). Thus, there is no suggestion that the fatty acids used to make the salts in this category are genotoxic. The genotoxicity of lithium compounds has been tested and reviewed (Léonard et al., 1995). The overall evidence from several *in vitro* and *in vivo* studies with soluble lithium salts indicated no mutagenic activity and a possible effect on chromosomes only after a very high intraperitoneal dose of lithium citrate (2 g/kg).

**Summary:** Based on the low solubility of the lithium salts of fatty acids in this category and the existing data on lithium, **no additional testing is proposed.**

**Reproductive Toxicity**

No studies have been reported on the reproductive toxicity of calcium salts of fatty acids in this category. The toxicity of a vehicle containing 5.5 percent magnesium stearate was tested orally in pregnant rabbits at a dose of 2.5 mg/kg (Gottschewski, 1967). There were no teratogenic effects.

No studies have been reported on the reproductive toxicity of lithium salts of fatty acids in this category.

Rats and mice were fed diets containing up to 10 percent castor oil for 13 weeks. No significant effects were observed in screening for male reproductive endpoints or length of female estrous cycles (NTP, 1992).

**Summary:** Since the fatty acids used to make the salts in this category are edible themselves or closely related to edible fats and oils, hazard characterization is focused on the metal ions, calcium and lithium. Relative to two of the calcium salts in the category, it has been concluded that the “Chemical is not considered a candidate for testing under the HPV Challenge Program, based on preliminary EPA review indicating that testing using the SIDS base set would not further our understanding of this chemical’s properties” (EPA HPV Challenge Program Chemical List). **Therefore, no testing of other calcium salts is proposed.**

The use of partially soluble lithium salts in the treatment of psychological conditions has been associated with developmental toxicity. A causative relationship has not been established between the ingestion of lithium pharmaceuticals and birth defects. This issue was reviewed by Léonard, et al. (1995). The lithium salts in this category are not considered soluble. However,

given the lack of data for the lithium salts of fatty acids in this category, **the HPV Test Group proposes to conduct a dermal reproductive/developmental study on a grease with a lithium fatty acid salt as thickener.**

## EVALUATION OF PHYSICOCHEMICAL AND ENVIRONMENTAL FATE DATA AND PROPOSED TESTING

Although some data for products in this category exist, not all of these endpoints are defined and a consensus database for chemicals that represent products in this category does not exist. Therefore, calculated and measured representative data will be identified and a technical discussion provided, where appropriate. The EPIWIN© computer model, as discussed in the US EPA document entitled "The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program", will be used to calculate physical-chemical properties of substances in this category (U.S. EPA, 2000).

A fundamental characteristic of the fatty acid salts in this category is that in their pure form, they dissociate into the free metal and fatty acid anion. The pH of an aqueous solution of these substances where 50% of the total substance is in the molecular and dissociated forms is termed its pKa. A lowering of the pH relative to its pKa shifts the equilibrium to the dissociated forms, while raising the pH relative to its pKa shifts the equilibrium to the molecular form. Therefore, knowing the pKa of a substances provides information on the predominant form of the molecule at a given pH. While pKa values for the specific substances in this category were not available, pKa values of analogous metal salts of fatty acids were found to be circumneutral, meaning that the salt and free acid anion would both exist at typical environmental pHs. When measured data cannot be found for substances in this category, data for similar compounds and modeled data will be used to describe the physicochemical and environmental fate endpoints.

### Physicochemical Data

Grease thickeners included in this category are all calcium or lithium salts of fatty acids. The hydrocarbon chain lengths vary from nine carbon atoms (nonanedioic acid, dilithium salt; CAS No. 38900-29-7) to greater than 18 (hydrocarbon waxes, oxidized, methyl esters, calcium salts, CAS NO. 68603-11-2, and fatty acids, $C_{16-22}$, lithium salts, CAS No. 68783-36-8). For the HPV endpoints, melting point, boiling point, vapor pressure, partition coefficient and water solubility, a technical discussion will be prepared in the robust summary that describes these endpoints. Measured data on specific structures in this category will be provided, when available, and

supplemented with physicochemical data of chemically similar analogs as well as estimates made using the EPIWIN© model (EPA 2000).

**Summary: A technical discussion for melting point, boiling point, vapor pressure, partition coefficient and water solubility will be developed** for the robust summary that includes measured data on specific category structures, chemical analogs, and estimated properties made by EPIWIN© (EPA 2000).

**Environmental Fate Data**

Environmental fate endpoints include biodegradation, photodegradation, hydrolysis, and distribution in the environment (fugacity). The physicochemical properties and molecular structure of a chemical will influence the degradation processes it may be subjected to in the environment, as well as the way in which it partitions among environmental compartments (e.g., air, water, soil, sediment).

Because the compounds in this category are made from fatty acids that are either edible or similar in structure to edible fatty acids, the hydrocarbon moieties of the calcium or lithium salts are expected to be inherently biodegradable in the environment. Free calcium or lithium resulting from the dissociation of the salt would be expected to engage in chemical reactions with other naturally occurring anions in a manner predictable within the thermodynamic boundaries specific to these cations. No biodegradation data were available for the materials in this category. However, these substances are similar to other fatty acids used in the food and cosmetic industry. Therefore, a technical discussion on the potential for these substances to undergo biodegradation will be developed in the robust summary. Data from chemically similar analogs of fatty acids and/or degradation estimates will be presented in the technical discussion.

For the photodegradation endpoint, estimates of the atmospheric oxidation potential will be calculated. Hydrolysis is not a relevant endpoint for substances in this category because these materials do not contain chemical linkages subject to hydrolysis.

Equilibrium models are used to calculate chemical fugacity that can provide information on where a chemical is likely to partition in the environment. These data are useful in identifying environmental compartments that could potentially receive a released chemical. Fugacity data can only be calculated. In its guidance document for HPV data development, the U.S. EPA states that it accepts Level I fugacity data as an estimate of chemical distribution values. The input data required to run a Level I model include basic physicochemical parameters;

distribution is calculated as percent of chemical partitioned to different environmental compartments within a standardized regional environment. Level I data are basic partitioning data that allow for comparisons between chemicals and indicate the compartment(s) to which a chemical is likely to partition in the environment. Estimates of the percent distribution for various environmental media (i.e., soil, water, air, sediment, biota) will be done and included in the robust summary.

**Summary: No testing is proposed**. The robust summary will provide technical discussions of the environmental fate characteristics (biodegradation, photodegradation, hydrolysis and fugacity) of substances in the grease thickeners category.

## EVALUATION OF ECOTOXICITY AND PROPOSED TESTING

As explained in the category description above, the grease thickening agents Included in this category do not exist outside of the grease matrix, but are synthesized within the grease by adding one of several varieties of fatty acids (e.g., stearic acid, ricinoleic acid – i.e., castor oil, etc.) and metal hydroxides (e.g., lithium, calcium) to mineral oil. The caustic and fatty acid react within the mineral oil medium to form an insoluble metal salt of the fatty acid. Such "in situ" generation of the thickening agents limits the potential for environmental exposure to these substances since they are entrained within the grease matrix. In addition, fatty acids and metals used in these products are resistant to "bleed-out" (i.e., disperse from the grease matrix), as this would compromise the performance characteristics of the grease.

**Summary: No testing is proposed**. A technical discussion will be prepared for the robust summary that describes the potential for substances in this category to affect aquatic organisms. Aquatic toxicity data on the dissociation products of these grease thickeners, i.e., lithium, calcium, and the associated fatty acids will be included in the discussion along with relevant physicochemical information affecting their distribution in aquatic environments.

**TABLE 3. MATRIX OF AVAILABLE ADEQUATE DATA AND PROPOSED TESTING FOR SELECTED TEST MATERIAL**

| Test | Calcium salts in this category | Li 12-hydroxy stearate in grease | Other lithium salts in this category |
|---|---|---|---|
| Melting Point | Model | Model | Model |
| Boiling Point | Model | Model | Model |
| Vapor Pressure | Model | Model | Model |
| Partition Coefficient | Model | Model | Model |
| Water Solubility | Model | Model | Model |
| Photodegradation | Model | Model | Model |
| Stability in Water | TD | TD | TD |
| Transport and Distribution | Model | Model | Model |
| Biodegradation | TD | TD | TD |
| Acute Toxicity to Fish | TD | TD | TD |
| Acute Toxicity to Aquatic Invertebrates | TD | TD | TD |
| Toxicity to Algae | TD | TD | TD |
| Acute Toxicity | Adequate | Adequate | Read-across [C] |
| Repeated Dose | Adequate | Adequate | Read-across [C] |
| Genotoxicity, *in vitro* | Adequate | Read-across [C] | Read-across [C] |
| Genotoxicity, *in vivo* | Adequate | Read-across [C] | Read-across [C] |
| Repro/ Developmental | Adequate | **TEST** | Read-across [C] |

Adequate Indicates adequate existing data.
Test Indicates proposed testing
Model Indicates data will be obtained with EPA approved models
C Indicates category read-across from existing or proposed test data
TD Technical discussions will be developed to address these endpoints as appropriate.

There are no studies available on the developmental and reproductive toxicity of the lithium salts of fatty acids in the Grease Thickeners Category. Therefore, this study plan proposes

reproductive/ developmental toxicity screening tests in rats (OECD 421) by the dermal route of exposure. Dermal exposure was selected as the route by which humans are most likely to come in contact with the salts of fatty acids in greases. The test material will be a grease made from USP white mineral oil thickened with approximately 12% lithium 12-hydroxystearate. No additives will be present. A lithium salt thickened grease was selected because, in the lubricant industry, the salts of fatty acids are only produced *in situ* in the mineral oil matrix, and are not synthesized separately.

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**ROBUST SUMMARY**
**OF INFORMATION ON**

**Substance Group** **GREASE THICKENERS**



**Summary prepared by** American Petroleum Institute

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NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

# 1. General Information

**Id** Greases
**Date** 12. 24 .2003

### 2.1 MELTING POINT

**Method** : Calculated by MPBPWIN, V1.40 subroutine in EPIWIN V3.10 computer model (EPA 2000)
**GLP** : No
**Test Substance** :
**Remark** : The members of the grease thickeners category are composed of various salts of fatty acids containing hydrocarbon chains of 9 to 22 carbon atoms. The melting point estimates given here are for fatty acid salts covering this range of carbon atoms. The data represent a potential melting point range for all substances in the grease thickeners category.

**Result** :

| | Molecular Weight | No. C Atoms | Estimated MP Value, °C |
|---|---|---|---|
| **Lithium Salts** | | | |
| nonanedioic acid, dilithium salt | 200.09 | 9 | 186 |
| octadecanoic acid, lithium salt | 290.42 | 18 | 249 |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | 306.42 | 18 | 264 |
| docosanoic acid, lithium salt | 346.53 | 22 | 271 |
| **Calcium Salts** | | | |
| octadecanoic acid, 12-hydroxy, calcium salt | 639.03 [1] | 36 | 320 |
| stearic acid, calcium salt | 607.04 [1] | 36 | 288 |

[1] Compound composed of two fatty acid molecules attached to calcium.

**Reliability** : (2) Reliable with restrictions
Estimated melting points were calculated using a validated computer model.
: (17)

### 2.2 BOILING POINT

**Method** : Calculated by MPBPWIN, V1.40 subroutine in EPIWIN V3.10 computer model (EPA 2000)
**GLP** : No
**Test Substance** :
**Remark** : The members of the grease thickeners category are composed of various salts of fatty acids containing hydrocarbon chains of 9 to 22 carbon atoms. The boiling point estimates given here are for fatty acid salts covering this range of molecular weights and number of carbon atoms. The data represent a potential boiling point range for all substances in the grease thickeners category.

**Result** :

| | Molecular Weight | No. C Atoms | Estimated BP Value, °C |
|---|---|---|---|
| **Lithium Salts** | | | |
| nonanedioic acid, dilithium salt | 200.09 | 9 | 484 |
| octadecanoic acid, lithium salt | 290.42 | 18 | 578 |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | 306.42 | 18 | 611 |
| docosanoic acid, lithium salt | 346.53 | 22 | 624 |
| **Calcium Salts** | | | |
| octadecanoic acid, 12-hydroxy, calcium salt | 639.03 [1] | 36 | 730 |
| stearic acid, calcium salt | 607.04 [1] | 36 | 661 |

[1] Compound composed of two fatty acid molecules attached to calcium.

**Reliability** : (2) Reliable with restrictions
Estimated melting points were calculated using a validated computer model.

: (17)

## 2.3 VAPOUR PRESSURE

**Method** : Calculated by MPBPWIN, V1.40 subroutine in EPIWIN V3.10 computer model (EPA 2000)

**GLP** : No

**Test Substance** :

**Remark** : The members of the grease thickeners category are composed of various salts of fatty acids containing hydrocarbon chains of 9 to 22 carbon atoms. The vapour pressure estimates given here are for fatty acid salts covering this range of molecular weights and number of carbon atoms. The data represent a potential vapour pressure range for all substances in the grease thickeners category.

**Result** :

| | Molecular Weight | No. C Atoms | Estimated VP Value, hPa |
|---|---|---|---|
| **Lithium Salts** | | | |
| nonanedioic acid, dilithium salt | 200.09 | 9 | $2 \times 10^{-9}$ |
| octadecanoic acid, lithium salt | 290.42 | 18 | $1 \times 10^{-12}$ |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | 306.42 | 18 | $2 \times 10^{-16}$ |
| docosanoic acid, lithium salt | 346.53 | 22 | $5 \times 10^{-14}$ |
| **Calcium Salts** | | | |
| octadecanoic acid, 12-hydroxy, calcium salt | 639.03 [1] | 36 | $1 \times 10^{-21}$ |
| stearic acid, calcium salt | 607.04 [1] | 36 | $6 \times 10^{-14}$ |

[1] Compound composed of two fatty acid molecules attached to calcium.

**Reliability** : (2) Reliable with restrictions
Estimated melting points were calculated using a validated computer model.

: (17)

### 2.4 PARTITION COEFFICIENT

**Method** : Calculated by MPBPWIN, V1.40 subroutine in EPIWIN V3.10 computer model (EPA 2000)
**GLP** : No
**Test Substance** :
**Remark** : Because fatty acids are ionizable compounds, Kow measurements (hence log P) can vary greatly with pH. The variation depends upon pH and the pKa of the compound. In general, Kow values of a compound are lower when it exists predominantly in the ionized form as compared to existing primarily in the non-ionized form. The KOWWIN V1.66 model handles ion pairs in a special way and gives Kow estimates that are an estimate for the ionized acid. Many fatty acids have pKa values circumneutral, and they would exist predominantly in the molecular form at environmentally relevant pHs. Therefore, the estimates given here are potentially lower than what would be expected for the salt form at typical environmental pHs.

**Result** :

| | Molecular Weight | No. C Atoms | Estimated Log Kow |
|---|---|---|---|
| **Lithium Salts** | | | |
| nonanedioic acid, dilithium salt | 200.09 | 9 | -3.56 |
| octadecanoic acid, lithium salt | 290.42 | 18 | 4.13 |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | 306.42 | 18 | 2.60 |
| docosanoic acid, lithium salt | 346.53 | 22 | 6.10 |
| **Calcium Salts** | | | |
| octadecanoic acid, 12-hydroxy, calcium salt | 639.03 [1] | 36 | 11.7 |
| stearic acid, calcium salt | 607.04 [1] | 36 | 14.3 |

[1] Compound composed of two fatty acid molecules attached to calcium.

**Reliability** : (2) Reliable with restrictions
Estimated melting points were calculated using a validated computer model.
: (17)

### 2.6.1 SOLUBILITY IN DIFFERENT MEDIA

**Solubility in** : Water
**Method** : Calculated by MPBPWIN, V1.40 subroutine in EPIWIN V3.10 computer model (EPA 2000)
**GLP** : No
**Test Substance** :
**Remark** : The members of the grease thickeners category are composed of various salts of fatty acids containing hydrocarbon chains of 9 to 22 carbon atoms. The water solubility estimates given here are for fatty acid salts covering this range of molecular weights and number of carbon atoms. The data represent a potential water solubility range for all substances in the grease thickeners category.

**Result** :

| | Molecular Weight | No. C Atoms | Estimated Solubility, mg/l |
|---|---|---|---|
| **Lithium Salts** | | | |
| nonanedioic acid, dilithium salt | 200.09 | 9 | $1 \times 10^{6}$ |
| octadecanoic acid, lithium salt | 290.42 | 18 | 4.1 |
| octadecanoic acid, 12-hydroxy-stearate, lithium salt | 306.42 | 18 | 0.1 |
| docosanoic acid, lithium salt | 346.53 | 22 | 0.04 |
| **Calcium Salts** | | | |
| octadecanoic acid, 12-hydroxy, calcium salt | 639.03 [1] | 36 | $9.7 \times 10^{-9}$ |
| stearic acid, calcium salt | 607.04 [1] | 36 | $8.2 \times 10^{-11}$ |

[1] Compound composed of two fatty acid molecules attached to calcium.

**Reliability** : (2) Reliable with restrictions
Estimated melting points were calculated using a validated computer model.

: (17)

### 3.1.1 PHOTODEGRADATION

### 3.1.2 STABILITY IN WATER

### 3.1.3 STABILITY IN SOIL

### 3.2.1 MONITORING DATA

### 3.2.2 FIELD STUDIES

### 3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

### 3.3.2 DISTRIBUTION

### 3.4 MODE OF DEGRADATION IN ACTUAL USE

### 3.5 BIODEGRADATION

**4.1 ACUTE/PROLONGED TOXICITY TO FISH**

**4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES**

**4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE**

**4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA**

**4.5.1 CHRONIC TOXICITY TO FISH**

**4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES**

**4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS**

**4.6.2 TOXICITY TO TERRESTRIAL PLANTS**

**4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS**

**4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES**

**4.7 BIOLOGICAL EFFECTS MONITORING**

**4.8 BIOTRANSFORMATION AND KINETICS**

**4.9 ADDITIONAL REMARKS**

### 5.1.1 ACUTE ORAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | > 5000 mg/kg bw |
| **Species** | : | Rat |
| **Strain** | : | Sprague-Dawley |
| **Sex** | : | Male/female |
| **Number of animals** | : | 10 |
| **Vehicle** | : | Undiluted |
| **Doses** | : | 5000 mg/kg only |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | Grease Starplex 2<br>Starplex 2 is a grease with the following composition<br><br>Wt % base oil ~65<br>Thickeners<br>Li 12-hydroxy stearate 13.1%<br>Dilithium azelate 2.6%<br>Wt % other additives ~20 |
| **Method** | : | Five male and five female fasted rats were given a single oral dose (5000 mg/k) of the test material. The rats were observed 1, 4 and 24 hours after administration of the test material for clinical signs of toxicity and any other pharmacological signs. Body weights were recorded before administration of the test material and again on days 7 and 14.<br>All animals were sacrificed on day 14 and a gross necropsy was performed on each of them. Abnormal observations were recorded. |
| **Result** | : | No clinical signs were observed and no animal died during the study.<br>There was a body weight increase for all animals on the study. At necropsy there were no abnormal observations.<br>The $LD_{50}$ of the test material was greater than 5000 mg/kg. |
| **Reliability** | : | (1) valid without restriction |

(12)

| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | > 10000 mg/kg bw |
| **Species** | : | Rat |
| **Strain** | : | Albino |
| **Vehicle** | : | Corn oil |
| **Doses** | : | 0.05-10.0 g/kg |
| **Year** | : | 1982 |
| **GLP** | : | No data |
| **Test substance** | : | Magnesium stearate |
| **Result** | : | The publication states:<br>Given as 25% suspension in corn oil.<br><br>Animals fasted overnight and then given dose ranging from 0.05 to 10.0 g/kg. Animals observed daily for 14 days. All animals at 10.0 g/kg exhibited mild diarrhea. |
| **Reliability** | : | (4) not assignable<br>Information is taken from the report of a Cosmetic ingredient review panel.<br>Original data not available. |

(2)

| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | 5000 - 15000 mg/kg bw |
| **Species** | : | Rat |
| **Strain** | : | Albino |
| **Vehicle** | : | Propylene glycol |
| **Doses** | : | 0.05, 1, 3 & 15 g/kg |
| **Year** | : | 1982 |
| **GLP** | : | No data |
| **Test substance** | : | Lithium stearate |
| **Result** | : | Lithium stearate was administered in propylene glycol (concentration unspecified) to 30 albino rats (sex not specified).<br><br>The publication states:<br><br>Animals fasted for 24 hrs. and then given dosages ranging from 0.05 to 15.0 g/kg. Animals dosed at 0.05, 1.0 and 3.0 g/kg showed no toxic effects; all animals administered 15.0 g/kg died within 16 hrs. having exhibited unkempt coats, impaired locomotion and lethargy prior to death. |
| **Reliability** | : | (4) not assignable<br>Information is taken from the report of a Cosmetic ingredient review panel. Original data not available. |

(2)

### 5.1.3 ACUTE DERMAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | > 3000 mg/kg bw |
| **Species** | : | Rabbit |
| **Strain** | : | New Zealand white |
| **Sex** | : | Male/female |
| **Number of animals** | : | 10 |
| **Vehicle** | : | Undiluted |
| **Doses** | : | 300 mg/kg |
| **Method** | : | |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | Grease Starplex 2<br>Starplex 2 is a grease with the following composition<br><br>Wt % base oil ~65<br>Thickeners<br>Li 12-hydroxy stearate 13.1%<br>Dilithium azelate 2.6%<br>Wt % other additives ~20 |
| **Method** | : | Undiluted test material was applied to the shorn dorsal skin of five male and five female NZW rabbits. The applied grease was covered with an occlusive dressing which was left in place for 24 hours. Following the 24 hours exposure period the covering was removed and any residual test material was wiped from the skin using mineral oil and a gauze. Observations were recorded daily throughout the following 14 days. Body weights were recorded prior to application of the test material and again on days 7 and 14. All rabbits were killed by lethal injection and a gross necropsy was performed and a record made of any abnormalities. |
| **Result** | : | There were no clinical signs of toxicity during the study and no animals |

died. Erythema and edema was observed at the treated skin site when the occlusive covering was removed. At this time average erythema and edema scores were 2.6 and 2 respectively (same average scores for each sex). The skin responses gradually subsided and by day 6 had completely disappeared. Animals gained weight during the study and no abnormalities were observed at necropsy.
The dermal $LD_{50}$ was therefore greater than 3000 mg/kg.

**Reliability** : (1) valid without restriction

(11)

### 5.2.1 SKIN IRRITATION

**Species** : Rabbit
**Concentration** : Undiluted
**Exposure** : Semi occlusive
**Exposure time** : 4 hour(s)
**Number of animals** : 6
**Vehicle** : None
**Year** : 1944
**GLP** : Yes
**Test substance** : Grease Starplex 2
Starplex 2 is a grease with the following composition

| | |
|---|---|
| Wt % base oil | ~65 |
| Thickeners | |
| Li 12-hydroxy stearate | 13.1% |
| Dilithium azelate | 2.6% |
| Wt % other additives | ~20 |

**Method** : 0.5 ml of undiluted test material was applied to three separate sites on the shorn dorsal trunks of three male and three female NZW rabbits. Each site was covered with a semi occlusive dressing. One site was abraded, the other two were intact skin.
One of the intact skin sites was only covered for 4 hours and the other two sites were covered for 24 hours. At the end of the exposure periods, residual test material was removed from the skin using gauze and mineral oil.
After patch removal, the test site was examined for erythema and edema and the responses were scored immediately using the standard Draize scale. Skin responses were scored again at 1, 24, 48 and 72 hours after patch removal and again on days 4 through 6.
Body weights of animals were recorded before application of test material and again at the end of the study.

**Result** : No clinical signs of toxicity were observed and all animals gained weight over the course of the study.
Average scores for erythema and edema are as shown in the following table.

| **Time** | **4 hour** | | **24 hour exposure** | | | |
|---|---|---|---|---|---|---|
| | **Erythema** | **Edema** | **Erythema** | | **Edema** | |
| | | | I* | A | I | A |
| 0 hrs | 0.7 | 0 | 3.2 | 3.2 | 2.7 | 2.8 |
| 1 hr | 0.7 | 0 | 3.2 | 3.2 | 2.7 | 2.8 |
| 24 hrs | 0.2 | 0.2 | 3 | 3.2 | 2.3 | 2.3 |
| 48 hrs | 0.2 | 0.2 | 2 | 2.2 | 1.7 | 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 72 hrs | 0.2 | 0 | 1.5 | 1.7 | 1.3 | 1.5 |
| Day 4 | 0 | 0 | 1 | 1 | 0.5 | 0.7 |
| Day 5 | | | 0.2 | 0.2 | 0 | 0 |
| Day 6 | | | 0 | 0 | 0 | 0 |

* I = Intact, A = Abraded

The four hour exposures resulted in only slight irritation which had cleared by day 4.

24 hour exposure caused moderate to severe erythema with well defined to severe edema. Skin responses had cleared by day 6 and there was no evidence that abraded skin was more irritated than intact skin.

The calculated Primary irritation indices were:
4 hour exposure 0.38
24 hour exposure 4.92

**Reliability** : (1) valid without restriction

(13)

**Species** : Rabbit
**Concentration** : Undiluted
**Exposure time** : 4 hour(s)
**Number of animals** : 6
**Vehicle** : None
**PDII** : 0
**Result** : Not irritating
**Year** : 1982
**GLP** : No data
**Test substance** : Magnesium stearate

**Method** : Two studies were summarized:
A four hour study of acute dermal corrosion and a 24 hour study for skin irritation.
In both studies 6 albino rabbits were used.
The test material was applied under an occlusive dressing in both studies.
Also in both studies half the test sites were abraded while the other half were intact skin.

The corrosion study was conducted according to the procedure described in 49 CFR 173.240 (a) (1).

**Result** : The primary irritation index in both studies was 0.
**Reliability** : (4) not assignable
Information is taken from the report of a Cosmetic ingredient review panel.
Original data not available.

(2)

### 5.2.2 EYE IRRITATION

**Species** : Rabbit
**Concentration** : Undiluted
**Dose** : 0.1 ml
**Number of animals** : 6
**Vehicle** : None
**Year** : 1994
**GLP** : Yes

**Test substance** : Grease Starplex 2
Starplex 2 is a grease with the following composition

| | |
|---|---|
| Wt % base oil | ~65 |
| Thickeners | |
| Li 12-hydroxy stearate | 13.1% |
| Dilithium azelate | 2.6% |
| Wt % other additives | ~20 |

**Method** : 0.1 ml of test material was placed in the conjunctival sac of the right eye of six female NZW rabbits. The left eye was untreated and served as control. The eyes were examined at 1, 24, 48 and 72 hours after treatment and again on day 7. Ocular reactions were scored according to the standard Draize scale.
Body weights were recorded at the beginning and the end of the study.

**Result** : Conjunctival redness was observed in all animals 1 hour after application of the test material and in three animals at 24 hours. This conjunctival response continued in one animal for 72 hours but was not seen in any animal after 7 days.
Iritis was observed in only one animal at 24 hours and corneal opacity also occurred at 24 hours in the same animal and this persisted for 24 hours. All eyes were normal after 7 days.

The average Draize scores for 6 rabbits are shown in the following table.

| Time after application of test material | Cornea | Iris | Conjunctivae |
|---|---|---|---|
| 1 hour | 0 | 0 | 10 |
| 24 hours | 0.8 | 0.8 | 3.3 |
| 48 hours | 0.8 | 0 | 2.7 |
| 72 hours | 0 | 0 | 1.3 |
| 7 Days | 0 | 0 | 0 |

**Reliability** : (1) valid without restriction

(14)

**Species** : Rabbit
**Concentration** : Undiluted
**Comment** : Not rinsed
**Number of animals** : 6
**Vehicle** : None
**Result** : Not irritating
**Year** : 1982
**GLP** : No data
**Test substance** : Magnesium stearate

**Result** : The scores were zero on days 1, 2 and 3
**Reliability** : (4) not assignable
Information is taken from the report of a Cosmetic ingredient review panel. Original data not available.

(2)

## 5.3 SENSITIZATION

**Type** : Buehler Test
**Species** : Guinea pig
**Concentration** : 1st: Induction undiluted occlusive epicutaneous
2nd: Induction undiluted occlusive epicutaneous
3rd: Induction undiluted occlusive epicutaneous
**Number of animals** : 10
**Vehicle** : None
**Result** : Not sensitizing
**Year** : 1997
**GLP** : Yes
**Test substance** : Starplex MPGM
Starplex MPMG 2 is a grease with the following composition

| | |
|---|---|
| Wt % base oil | ~80 |
| Thickeners | |
| Li 12-hydroxy stearate | 8.8% |
| Dilithium azelate | 1.8% |
| Wt % other additives | ~10 |

**Method** : On the basis of the results of a preliminary irritation screen, it was decided to use undiluted test material for the induction and challenge dosing in the sensitization test.
The test material was applied under a Hilltop chamber to the shorn skin of 10 male and 10 female Guinea pigs. The patches were allowed to remain in place for six hours, after which they were removed and any residual test material was also removed from the skin using a gauze and mineral oil.
The treated sites were examine after each dosing day and scored for dermal irritation at 24 and 48 hours. This dosing and scoring procedure was performed once a week for three weeks.
A concurrent positive control group of five animals (3 males and 2 females) was treated with 0.3% 1-chloro-2,4-dinitrobenzene in 80% ethanol (ethanol in distilled water).
An additional group of ten animals (5 of each sex) was treated with vehicle (mineral oil).

Fourteen days after the last induction dose, the animals were challenged by applying material in the same manner as the induction applications but on a naive site.
The vehicle control group was challenged with mineral oil and test substance.
The positive control group animals were challenged with DNCB at 0.01% and 0.2% in acetone.
All animals were observed for local and systemic effects.
24 hours after challenge, the animals were depilated. After a minimum of 2 hours following depilation the test sites were assesed and graded (24 hour grade) and were graded again after a further 24 hours (48 hour grade).

When skin reactions were graded throughout the study scores were attributed to each test site on a scale of 0-3 for erythema.
After the sensitization doses a score of 1 or more was taken to indicate that sensitization had occured. Furthermore if the test reactions exceeded the most severe control reactions, the animal was considered to be sensitized.

**Result** : A summary of the challenge scores is given in the following table.

| **Test** | **% animals with score at 24 hours** | | | | |
|---|---|---|---|---|---|
| **Group** | **0** | **+** | **1** | **2** | **3** |
| Vehicle control Induced with mineral oil | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Mineral oil challenge | 100 | 0 | 0 | 0 | 0 |
| Test material challenge | 100 | 0 | 0 | 0 | 0 |
| Test material induced with neat test material | | | | | |
| Test material challenge | 100 | 0 | 0 | 0 | 0 |
| Positive control animals induced with 0.3% DNCB | | | | | |
| 0.01% DNCB challenge | 60 | 20 | 20 | 0 | 0 |
| 0.2% DNCB challenge | 0 | 0 | 20 | 0 | 80 |

The positive control data clearly demonstrate the sensitivity of the test method. The test material itself did not cause skin sensitization in this study.

**Test substance** :
**Reliability** : (1) valid without restriction

(15)

## 5.4 REPEATED DOSE TOXICITY

**Type** : Sub-chronic
**Species** : Rat
**Sex** : Male/female
**Strain** : Wistar
**Route of admin.** : Oral feed
**Exposure period** : 3 Months
**Frequency of treatm.** : Daily in the diet
**Doses** : 5, 10 & 20% in the diet
**Control group** : Yes
**NOAEL** : 5 %
**Year** : 1980
**GLP** : No data
**Test substance** : Magnesium stearate

**Method** : Groups of 20 male and 20 female six week old rats were fed diets containing 5, 10 or 20 magnesium stearate. The diets were semi synthetic in which sodium caseinate replaced casein. The carbohydrates of the diet were substituted by magnesium stearate as follows:

| Group | Magnesium stearate % in diet | Carbohydrate % in diet |
|---|---|---|
| Contro l | 0 | 67.3 |
| | 5 | 62.3 |
| | 10 | 57.3 |
| | 20 | 47.8 |

The diets fed were considered isocaloric, as stearate has a calorific value of about 9, and a pilot study demonstrated that 35-40% of the stearate is absorbed at a 10% level in the diet. Acidified water (pH 3.5) was available ad libitum.
The animals were weighed once weekly and food utilization and weight gain was calculated for each sex of all groups of rats.
Blood samples were taken from 8 males and 8 females from each group prior to dosing and at 8 and 12 weeks. The following hematological and clinical chemistry determinations were made:

Hematology
Hemoglobin
packed cell volume (PCV)
red cell count

total white cell count
reticulocyte count
differential white cell count.

Clinical chemistry
Glucose
urea
aspartate amino transferase
alkaline phosphatase
At the termination of the study, the rats were sacrificed and the following organs were weighed: thymus, liver, kidneys, adrenals, testes/ovaries, heart, lungs, brain and pituitary.
Samples of the organs listed above and the following tissues were taken for light microscopy: urinary bladder, stomach, duodenum, pancreas, jejunum, cecum, colon, thyroid, parathyroid, triceps, brachial muscle, ischiadic nerve, axillar lymph node, uterus, sternum, eye, Harderian gland, skin and submandibular gland. Microscopic examination was undertaken on the high dose and control animals only.

**Result** **:** The weight gains of the 20% males were significantly less than he corresponding controls during the first 8 weeks of the study [No actual data given in the publication]. Concomitantly these animals were quiet with slow and unsteady movements. Four males in his group died within the first 2 months and all had stone formation in the lower urinary pathways and the deaths were considered to be related to this finding. One other male in this group was incontinent. In the remaining males, the symptoms receded during the following 4 weeks. There were no clinical effects in females in any group.
A reduction in PCV [P<0.01, but no data provided] was found in the 20% males compared to controls. No other hematological differences were reported.
In addition to the findings reported in the males that died in the 20% group, changes were also found in the renal pelvis and in the lower urinary pathways (due to stone formation) at autopsy in 4 males and one female in the 20 % group.
The relative liver and kidney weights recorded were as follows:

| **Dietary concentration** | **Sex** | **Liver g/100g body wt ±SD** | **Kidney g/100g body wt. ±SD** |
|---|---|---|---|
| 0 | M | 3.25±0.21 | 633±48.6 |
| 5 | M | 3.13±0.21* | 614±51.5 |
| 10 | M | 2.99±0.23*** | 599±40.6* |
| 20 | M | 2.82±0.18*** | 640±80.7 |
| | | | |
| 0 | F | 3.30±0.24 | 768±103 |
| 5 | F | 3.33±0.18 | 661±86.5*** |
| 10 | F | 3.31±0.31 | 667±54.0*** |
| 20 | F | 3.16±0.23* | 646±55.8*** |

* P< 0.05
*** P<0.001

Nephrocalcinosis was seen in all females and in 12/20 males in the control group. In 18 of the females nephrocalcinosis was regarded as severe. Slight to moderate nephrocalcinosis was observed in 19/20 of the females in the 20% group and 7/20 of the males were affected only slightly.

Deposition of iron was found in various amounts in kidney and in liver, the amount was increased in the liver of both sexes in the 20% group.
Liver glycogen showed a marked decrease in males in the 20% group and no difference was found in the females.

The authors comment that :

the occurrence of nephrocalcinosis is a common finding in animals fed semi-synthetic diets. The increased magnesium content of the diet could explain the reduction of nephrocalcinosis in the 20% animals. A high magnesium content of the diet has also been previously associated with a greater incidence of stone formation in the lower part of the urinary tract.

The authors concluded that:

when liver weight was used as a measure of adverse effect, the no effect level was estimated to be 5% magnesium stearate in the diet, corresponding to 2500 mg/kg body weight.

**Reliability** : (2) valid with restrictions
Few experimental details are provided and detailed results are not included in the publication.
However, the publication does provide useful information on the effects of repeated oral exposure to magnesium stearate.

(16)

**Type** : Sub-chronic
**Species** : Rat
**Sex** : Male/female
**Strain** : Sprague-Dawley
**Route of admin.** : Gavage
**Exposure period** : 90 days
**Frequency of treatm.** : Daily, seven days each week
**Doses** : 250, 500 & 1000 mg/kg/day
**Control group** : Yes, concurrent vehicle
**NOAEL** : 1000 mg/kg
**Year** : 1977
**GLP** : Yes
**Test substance** : R960002575
R960002575 is a code number for Starplex MPMG 2, which is a grease with the following composition

| | |
|---|---|
| Wt % base oil | ~80 |
| Thickeners | |
| Li 12-hydroxy stearate | 8.8% |
| Dilithium azelate | 1.8% |
| Wt % other additives | ~10 |

The test material was prepared as solutions in corn oil at the following concentrations to achieve the desired dose levels.

| Group | Dose group mg/kg/day | Concentration mg/ml | Volume ml/kg |
|---|---|---|---|
| I | 0 | 0 | 4 |
| II | 250 | 62.5 | 4 |
| III | 500 | 125 | 4 |
| IV | 1000 | 250 | 4 |

**Method** : Sprague-Dawley rats were used in this study. The animals (males and females) were aged 6 weeks at the beginning of the study.
The test material was administered orally by gavage at doses of 250, 500 or 1000 mg/kg/day in a dose volume of 4 ml/kg to groups of ten male and ten females for each dose level. Additionally, a group of ten male and ten females served as vehicle controls and for these corn oil alone (4ml/kg) was administered. This treatment was continued daily, seven days each week for 90 days.

Animals were observed twice daily for clinical signs of toxicity. A more thorough examination was undertaken weekly and this included a detailed physical examination for signs of local or systemic toxicity, pharmacological effects and palpation for tissue masses.
Body weights and food intakes were recorded weekly.
At the end of the study, on day 91, and after overnight fasting, animals were killed and blood samples were collected for the following hematological and serum chemistry investigations.

Hematology
Hemoglobin concentration
Hematocrit
Erythrocyte count
Platelet count
Reticulocyte count
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Prothrobin time
Activated partial thromboplastin time
Total and differential leukocyte counts
Erythrocyte morphology
Reticulocyte count

Clinical chemistry
Aspartate aminotransferase
Alanine aminotransferase
Alkaline phosphatase
Blood urea nitrogen
Fasting glucose
Total protein
Albumin
Globulin (calculated)
A/G ratio (calculated)
Creatinine
Total bilirubin
Sodium
Potassium
Chloride
Calcium
Inorganic phosphorus
Gamma-glutamyl transferase

A complete post mortem examination was performed on all animals. This included an examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

The following organs were weighed:
Adrenal glands, brain, kidneys, testes with epididymides, liver and ovaries.

The following tissues were preserved and processed for histological examination.
Adrenal glands (2)
Aorta
Bone (sternum/femur with articular surface)
Brain (medulla/pons, cerebrum and cerebellum)
Epididymis (2)
Esophagus
Eye with optic nerve*
Heart
Kidneys (2)
Large intestine (cecum, colon and rectum)
Lacrimal gland*
Liver (2 sections)
Lung with mainstem bronchi
Lymph node (mediastinal)
Lymph node (mesenteric)
Mammary gland*
Muscle (biceps femoris)*
Nasal turbinates
Nerve (sciatic)
Ovaries (2)
Pancreas
Pituitary
Prostate
Salivary gland (submaxillary)
Seminal vesicles
Skin (treated and untreated)
Small intestine ( duodenum, ileum and jejunum)
Spinal cord (cervical, thoracic, lumbar)*
Spleen
Stomach
Testes
Thymic region
Thyroid (with parathyroids)
Trachea
Urinary bladder
Uterus (body/horns with cervix)
Zymbal's gland*
Macroscopic lesions
Target organs
All the above tissues from all the animals in the high dose group and the controls were examined microscopically, except those indicated * which were preserved but not examined.

Statistical analysis
Body weight, body weight change fom week 0, food consumption, hematology and clinical chemistry parameters, terminal organ and body weights and organ/body weight ratios and organ/brain weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval.
A Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not,

nonparametric parametric procedures were used.

The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case, standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1% two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

**Result** : There were no mortalities during the study and there were no treatment-related clinical signs of toxicity. There were no adverse effects of treatment observed during the ophthalmoscopic examinations. Body weights were unaffected by treatment. The food consumption values for the 500 and 1000 mg/kg groups were often higher than the controls. However, they were considered to be within normal ranges and not treatment-related.

All except the following hematological parameters were unaffected by treatment. Those listed below were within the normal range for the laboratory and were not considered to be of toxicological significance.
Prothrombin time increases in males only:
15% in 500 mg/kg/day group
19% in 1000 mg/kg/day group
Activated partial thromboplastin time increase
18% in 250 and 1000 mg/kg/day groups

The only difference in clinical chemistry was a 9% increase in the phosphate levels of the 500 mg/kg/day females. This difference was not considered to be a treatment-related effect.

There were no effects on either organ weights, organ/body weight ratios or organ/brain weight ratios.

There were no macroscopic findings at necropsy and no treatment-related microscopic findings.

The NOAEL was considered to be 1000 mg/kg/day.

**Test substance** :
**Reliability** : (1) valid without restriction

(5)

**Type** : Sub-acute
**Species** : Rat
**Sex** : Male/female
**Strain** : Sprague-Dawley
**Route of admin.** : Dermal
**Exposure period** : Six hours daily
**Frequency of treatm.** : Daily, five days each week for four weeks
**Post exposure period** :

**Doses** : 525, 1050 & 2100 mg/kg/day
**Control group** : Yes, concurrent vehicle
**NOAEL** : 2100 mg/kg bw
**Method** :
**Year** : 1977
**GLP** : Yes
**Test substance** : TS: R960002575
R960002575 is a code number for Starplex MPMG 2, which is a grease with the following composition

| | |
|---|---|
| Wt % base oil | ~80 |
| Thickeners | |
| Li 12-hydroxy stearate | 8.8% |
| Dilithium azelate | 1.8% |
| Wt % other additives | ~10 |

The test material was prepared as solutions in corn oil at the following concentrations to achieve the desired dose levels.

| Group | Dose group mg/kg/day | Concentration mg/ml | Volume ml/kg |
|---|---|---|---|
| I | 0 | 0 | 4 |
| II | 250 | 62.5 | 4 |
| III | 500 | 125 | 4 |
| IV | 1000 | 250 | 4 |

**Method** : Male and female Sprague-Dawley rats aged approximately 7 and 9 weeks respectively were used in this study.
The test material was applied to the shorn skin of groups of five male and five females for each dose level. Additionally, a group of five male and five females served as vehicle controls and for these mineral oil alone was applied. The test sites were covered with an occlusive dressing which was left in place for six hours. After this time, the dressings were removed and any residual test material was removed from the skin using a gauze and mineral oil. This treatment was continued daily, five days each week for four weeks.

Animals were observed twice daily for clinical signs of toxicity. A more thorough examination was undertaken weekly and this included a detailed physical examination for signs of local or systemic toxicity, pharmacological effects and palpation for tissue masses.
Body weights and food intakes were recorded weekly.
At the end of the study, and after overnight fasting, animals were killed and blood samples were collected for the following hematological and serum chemistry investigations.

Hematology
Hemoglobin concentration
Hematocrit
Erythrocyte count
Platelet count
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Prothrobin time
Activated partial thromboplastin time
Total and differential leukocyte counts

Erythrocyte morphology
Reticulocyte count

Clinical chemistry
Aspartate aminotransferase
Alanine aminotransferase
Alkaline phosphatase
Blood urea nitrogen
Fasting glucose
Total protein
Albumin
Globulin (calculated)
A/G ratio (calculated)
Creatinine
Totall bilirubin
Sodium
Potassium
Chloride
Calcium
Inorganic phosphorus
Gamma-glutamyl transferase

A complete post mortem examination was performed on all animals. This included an examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

The following organs were weighed:
Adrenal glands, brain, kidneys, testes with epididymides, liver and ovaries.

The following tissues were preserved and processed for histological examination.
Adrenal glands (2)
Brain (medulla/pons, cerebrum and cerebellum)
Heart
Kidneys (2)
Liver (2 sections)
Ovaries (2)
Skin (treated and untreated)
Spleen
Testes with epididymides (2)

All the above tissues from all the animals in the high dose group and the controls were examined microscopically.

Statistical analysis
Body weight, body weight change from week 0, food consumption, hematology and clinical chemistry parameters, terminal organ and body weights and organ/body weight ratios and organ/brain weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval.
A Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not nonparametric parametric procedures were used.

The parametric procedures were the standard one way ANOVA using the F

distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case, standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1% two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

**Result** : All animals survived throughout the study and there were no clinical signs of toxicity and no dermal irritation was observed in the treatment groups.
Body weights were unaffected by treatment except that at four weeks the 2100 mg/kg/day males weighed approximately 3% less than the corresponding controls. However, this difference was not statistically significant.
Food consumption of the treatment groups were generally similar to the controls. A slight increase in food consumption of the mid dose males and high dose females at weeks one and two respectively were not considered to be of biological relevance.
Hematological and clinical chemical parameters, organ weights and microscopic findings were all unaffected by treatment.
It was concluded that the NOAEL was 2100 mg/kg/day.

**Reliability** : (1) valid without restriction

(6)

| | | |
|---|---|---|
| **Type** | : | Sub-chronic |
| **Species** | : | Rat |
| **Sex** | : | Male/female |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | Dermal |
| **Exposure period** | : | Six hours daily |
| **Frequency of treatm.** | : | Daily, five days each week for 13 weeks |
| **Doses** | : | 525, 1050 & 2100 mg/kg/day |
| **Control group** | : | Yes, concurrent vehicle |
| **NOAEL** | : | 2100 mg/kg |
| **Year** | : | 1997 |
| **GLP** | : | Yes |

**Test substance** : R960002575
R960002575 is a code number for Starplex MPMG 2, which is a grease with the following composition

| | |
|---|---|
| Wt % base oil | ~80 |
| Thickeners | |
| Li 12-hydroxy stearate | 8.8% |
| Dilithium azelate | 1.8% |
| Wt % other additives | ~10 |

The test material was prepared as solutions in mineral oil at the following concentrations to achieve the desired dose levels.

| Group | Dose group mg/kg/day | Concentration mg/ml | Volume ml/kg |
|---|---|---|---|
| I | 0 | 0 | 2.1 |
| II | 525 | 250 | 2.1 |
| III | 1050 | 500 | 2.1 |
| IV | 2100 | 1000 | 2.1 |

**Method** : Male and female Sprague-Dawley rats aged 7 and 9 weeks respectively were used in this study.
The test material was applied to the shorn skin of groups of ten male and ten females at doses of 525, 1050 or 2100 mg/kg/day. Additionally, a group of ten male and ten females served as vehicle controls and for these animals mineral oil alone was applied. The test sites were covered with an occlusive dressing which was left in place for six hours. After this time, the dressings were removed and any residual test material was removed from the skin using a gauze and mineral oil. This treatment was continued daily, five days each week for 13 weeks.

Animals were observed twice daily for clinical signs of toxicity. A more thorough examination was undertaken weekly and this included a detailed physical examination for signs of local or systemic toxicity, pharmacological effects and palpation for tissue masses. Examination of the skin for irritation was undertaken pre-test and then daily during the first week of exposure and weekly thereafter.
Body weights and food intakes were recorded weekly.
At the end of the study, and after overnight fasting, animals were killed and blood samples were collected for the following hematological and serum chemistry investigations.

Hematology
Hemoglobin concentration
Hematocrit
Erythrocyte count
Platelet count
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Prothrobin time
Activated partial thromboplastin time
Total and differential leukocyte counts
Erythrocyte morphology
Reticulocyte count

Clinical chemistry

Aspartate aminotransferase
Alanine aminotransferase
Alkaline phosphatase
Blood urea nitrogen
Fasting glucose
Total protein
Albumin
Globulin (calculated)
A/G ratio (calculated)
Creatinine
Total bilirubin
Sodium
Potassium
Chloride
Calcium
Inorganic phosphorus
Gamma-glutamyl transferase

A complete post mortem examination was performed on all animals. This included an examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

The following organs were weighed:
Adrenal glands, brain, kidneys, testes with epididymides, liver and ovaries.

The following tissues were preserved and processed for histological examination.
Adrenal glands (2)
Aorta
Bone (sternum/femur with articular surface)
Brain (medulla/pons, cerebrum and cerebellum)
Epididymis (2)
Esophagus
Eye with optic nerve*
Heart
Kidneys (2)
Large intestine (cecum, colon and rectum)
Lacrimal gland*
Liver (2 sections)
Lung with mainstem bronchi
Lymph node (mediastinal)
Lymph node (mesenteric)
Mammary gland*
Muscle (biceps femoris)*
Nasal turbinates
Nerve (sciatic)
Ovaries (2)
Pancreas
Pituitary
Prostate
Salivary gland (submaxillary)
Seminal vesicles
Skin (treated and untreated)
Small intestine ( duodenum, ileum and jejunum)
Spinal cord (cervical, thoracic, lumbar)*

Spleen
Stomach
Testes
Thymic region
Thyroid (with parathyroids)
Trachea
Urinary bladder
Uterus (body/horns with cervix)
Zymbal's gland*
Macroscopic lesions
Target organs
All the above tissues from all the animals in the high dose group and the controls were examined microscopically, except those indicated * which were preserved but not examined.

Statistical analysis
Body weight, body weight change from week 0, food consumption, hematology and clinical chemistry parameters, terminal organ and body weights and organ/body weight ratios and organ/brain weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval.
A Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not nonparametric parametric procedures were used.

The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case, standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1% two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

**Result** **:** There were no treatment-related deaths and there were no clinical signs of toxicity throughout the study. Although mild skin irritation was seen sporadically, it was not regarded as treatment-related. There were no treatment-related changes seen in the ophthalmoscopic examinations.
Apart from the mid dose males there were no treatment-related effects on body weight. In the case of the mid dose males, they were slightly lower than the controls throughout, but since animals in the higher dose group were unaffected this finding is not considered toxicologically significant.
Food consumption was unaffected by exposure to test material.
There were no biologically significant effects on either the hematology or clinical chemistry determinations that were undertaken.
Terminal organ weights, organ/body weight ratios and organ/brain weight ratios were unaffected by treatment.

There were no treatment-related macroscopic observations at necropsy and after histology, no microscopic changes were observed that were

considered to be treatment-related.

**Reliability** : (1) valid without restriction

(7)

**Type** : Sub-chronic
**Species** : Rat and Mouse
**Sex** : Male/female
**Strain** : Rat F344; Mouse B6C3F1
**Route of admin.** : oral feed
**Exposure period** : 90 days
**Frequency of treatm.** : Continual in the diet
**Doses** : 0.62, 1.25, 2.5, 5 & 10 % in the diet
**Control group** : Yes
**Year** : 1992
**GLP** : Yes
**Test substance** : Castor oil
USP AA grade castor oil was used.
It was incorporated in the diet and checks were made of actual dietary concentrations. These were as follows:

| Target concentration (%) | Actual concentration (%) |
|---|---|
| 0.62 | 0.62 |
| 1.25 | 1.26 |
| 2.5 | 2.64 |
| 5 | 4.91 |
| 10 | 9.67 |

**Method** : 10 animals of each sex and of each species were used for each dose group.
The treatment groups were fed diets containing 0.62, 1.25, 2.5, 5 or 10 % castor oil. In addition an extra 10 rats of each sex for each dietary level were fed for 21 days and these animals were used to provide blood samples for hematological and clinical chemical determinations on days 5 and 21, after which they were killed.
The main study animals were observed regularly throughout the study for clinical signs and were also weighed weekly. Food consumption was also recorded throughout the study.
At the end of the study at 13 weeks, all animals underwent a complete necropsy. Blood samples were taken for the following hematological and clinical chemical measurements.

Hematology: Red blood cell count, examination of red blood cell morphology, hematocrit, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, differential white cell count, reticulocyte count (absolute) and platelet count (absolute).

Clinical chemistry: alkaline phosphatase, albumin, urea nitrogen, creatinine, alanine aminotransferase activity, total bile acids, sorbitol dehydrogenase activity, total protein and creatinine kinase activity.

The following organs were weighed: liver, right kidney, right testicle, heart, thymus and lungs.

The following tissues were examined histopathologically in all control and

high dose rats and mice: Adrenal glands, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testis or ovaries/uterus, esophagus, eyes (if grossly abnormal), femur (including marrow), heart, jejunum, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pituitary gland, preputial or clitoral glands, rectum, salivary glands, skin, spinal cord and sciatic nerve (if neurological signs present), spleen, forestomach and glandular stomach, thymus, thyroid gland, trachea, urinary bladder, Zymbal glands, all gross lesions and tissue masses including lymph nodes.
In addition the livers from male rats of all other dose groups were examined.

Reproductive toxicity screen
Sperm motility and sperm density was assessed at necropsy.
Additionally for the 12 days prior to necropsy, females were subject to a vaginal lavage with saline. The aspirate was stained and examined to enable an assessment to be made of the stages of the estrous cycle.

Statistical analysis
Body weight and organ weight data were examined within each sex by one-way analysis of variance followed by Dunnett's t-test if pair-wise comparisons were indicated ($P<0.05$).

**Result** : The following is taken from the abstract of the report:

Exposure to castor oil at dietary concentrations as high as 10% in 13-week studies did not affect survival or body weight gains of rats or mice (10 per sex and dose). There were no biologically significant effects noted in hematologic analyses in rats. Mild increases in total bile acids and in serum alkaline phosphatase were noted at various times during the studies in rats receiving the higher dietary concentrations of castor oil. Liver weights were increased in male rats receiving the 10% dietary concentration and in male and female mice receiving diets containing 5% or 10% castor oil. However, there were no histopathologic lesions associated with these liver changes, nor were there any compound-related morphological changes in any organ in rats or mice. No significant changes were noted in a screening for male reproductive endpoints, including sperm count and motility, and no changes were observed in the length of estrous cycles of rats or mice given diets containing castor oil. Thus, no significant adverse effects of castor oil administration were noted in these studies.

**Reliability** : (1) valid without restriction

(10)

## 5.5 GENETIC TOXICITY 'IN VITRO'

**Type** : Ames test
**Result** : Negative

**Remark** : A cosmetic ingredients review panel concluded that magnesium stearate was not a mutagen in microbial tests with Salmonella typhimurium TA-1535, TA-1537, TA-1538 and Saccharomyces cerevisiae D4 with or without metabolic activation by liver and lung preparations from rats, mice and monkeys.
The panel cited the following as the sources of the information:
FASEB (1976) and Litton Bionetics (1976)

**Reliability** : (4) not assignable
Information taken from a review report. No actual data are given.
(3) (9)

## 5.7 CARCINOGENICITY

| | | |
|---|---|---|
| **Species** | : | Mouse |
| **Sex** | : | Male/female |
| **Strain** | : | C3H |
| **Route of admin.** | : | Dermal |
| **Exposure period** | : | 104 weeks |
| **Frequency of treatm.** | : | Twice weekly for 104 weeks |
| **Doses** | : | 50 mg/application |
| **Result** | : | Negative |
| **Control group** | : | Yes |
| **GLP** | : | Yes |
| **Test substance** | : | PARL-3093-GR-81<br>PARL-3093-GR-81 is the code number assigned to a sample of Molytex EP-2.<br>MolyteX EP-2 is a grease with the following composition |

| | | |
|---|---|---|
| Base oil | approx | 80% wt |
| Li 12-hydroxystearate | | 7.5% wt |
| Other additives | approx | 12% wt |

**Method** : 50 mg undiluted test material was applied twice weekly to the shorn interscapular region of 50 male and 50 female C3H mice aged 6-8 weeks. Positive control groups of 50 mice of each sex had 50 mg of a 0.05% solution of BaP in toluene applied twice weekly and these groups served as the positive controls. In addition solvent control groups of 50 mice of each sex received twice weekly applications of 50 mg toluene and a further group of 50 mice of each sex were untreated. The latter groups comprised the solvent and untreated controls respectively.

Applications were continued for 104 weeks or until a horny lesion on the surface of the skin grew to 1 $mm^3$. The lesion was diagnosed as a papilloma and the week that it appeared was recorded. If the tumor grew rapidly, invaded surrounding tissues, or became ulcerated and/or necrotic, it was diagnosed as an "advanced tumor" and the week of the transition was recorded. If a tumor regressed, treatment was resumed and continued until the end of the study or until another papilloma developed. If no growth appeared before death, the animal was recroded as not developing a tumor. If however, a second neoplasm developed, the time of its appearance was used in the calculation of the average latency period for the group.

Animals were observed daily throughout the study for clinical signs of toxicity.
At the termination of treatment, all surviving animals were sacrificed.
A complete post mortem examination was carried out on all animals sacrificed at the end of the study and on all animals that either died or were killed during the study because they were moribund.
At the post mortem examination the size and location of all skin neoplasms was recorded. Skin including the neoplasms and any other lesions was removed and placed in fixative for subsequent histopathological examination. Subcutaneous lymph nodes from the neck, ancillary region

and groin areas were also removed from the same animals and prepared for subsequent microscopic examination. The chest, abdominal and cranial cavities were examined and all organs were removed and a note made of their gross appearance. Tissues from each organ were preserved for possible microscopic examination.
H & E sections of the skin and of the mammary glands were examined microscopically.

**Result** : The number of mice with histologically-confirmed tumors is shown in the following table.

| No. Mice | No. mice with tumors Malignant | Benign | Latent period (weeks) |
|---|---|---|---|
| Untreated controls | | | |
| 46 males | 0 | 0 | - |
| 50 females | 1 | 2 | - |
| Toluene controls | | | |
| 48 males | 3 | 3 | 87 |
| 50 females | 5 | 2 | 72 |
| Grease | | | |
| 47 males | 0 | 2 | 67 |
| 50 females | 1 | 0 | 82 |
| BaP | | | |
| 46 males | 21 | 5 | 48 |
| 49 females | 45 | 2 | 49 |

It was concluded that the test material was not a skin carcinogen.

**Reliability** : (2) valid with restrictions
It should be noted that this study was a study of skin carcinogenicity only.

(1)

### 5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

**Species** : Rabbit
**Sex** : Female
**Route of admin.** : Gavage
**Frequency of treatm.** : Single dose given
**Doses** : 2.5 mg/kg
**Result** : Negative
**Year** : 1967
**GLP** : No
**Test substance** : Vehicle containing 5.5% Magnesium stearate
The test substance was a vehicle used to coat pharmaceutical tablets. The coating had the following composition:

| | |
|---|---|
| Polyethylene glycol | 27.5 mg |
| Starch | 34 mg |
| Talc | 27.5 mg |
| Silicon dioxide | 5.5 mg |
| Magnesium sulphate | 5.5 mg |

**Result** : The CIR report states:
Fourteen females received the vehicle per os at a dose of 2.5 mg/kg 70 hours post coitus whereas 13 females were given the same dose 192 hours post coitus. Compared with anomalies in the fetuses from 16 untreated mothers (12 of 112 offspring had anomalies) the vehicle containing 5.5% magnesium stearate induced anomalies in 9 out of 86 and 11 out of 90 fetuses respectively, thus demonstrating the absence of

teratogenic effect.

**Source** : Cosmetic Ingredient Panel review (1982)
**Reliability** : (4) not assignable
Information is taken from the report of a Cosmetic ingredient review panel. The material tested contained only 5.5% magnesium stearate and the method was inadequate for an evaluation of developmental toxicity.

(4)

**Species** : Various

**Remark** : Leonard et al reviewed information on the teratogenic effect of lithium compounds.
They comment that results have varied in intact animals. Whereas some authors have not demonstrated teratogenic effcects of lithium compounds, others have done so. The malformations reported have included reduced number and weight of the litter, more resorptions, wavy ribs and incomplete ossification.
These discrepancies might be due to a different sensitivity of the species and strains used, the stress of daily injections and/or differences in lithium concentrations present in serum during critical periods of development.

Lithium carbonate given to mice over several days yielding serum levels comparable to those in man treated for manic-depressive disorders did not show any effects, but six times higher doses caused malformations in the offspring. Chronic exposure to lithium at doses that produced serum levels of the same order as seen in patients was toxic but did not affect the entire litter nor was it teratogenic to individual embryos.

Many authors have reported that lithium causes congenital defects, especially of the cardiovascular system when given to women during the first trimester of pregnancy. As a result registers of "Lithium babies" have been set up. Up till now, analysis of the limited data have demonstrated an effect.

The authors conclude that the question of the possible teratogenicity of lithium remains open until further work is done.

**Reliability** : (4) not assignable

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